Protectiv Peptides Neurotoxin of C.Botulinum

This application is a continuation-in-part of U.S. patent Application 08/446,114, filed May 19, 1995, now U.S. Patent 6,287,566, issued September 11, 2001.

Field of the Invention:

This invention relates to immunization against toxic effect of neurotoxins of Clostridium bolutinum. Protective epitopes of the heavy chain of the neurotoxin of C. botulinum have been discovered. The invention also relates to preparation of protective immunotoxins of C. botulinum.

Background of the Invention:

Botulinum neurotoxin (BoNT) is one of the most potent toxins known to man. Ingestion or inhalation of toxin inhibits neurotransmitter release from synaptic vesicles, resulting in neuroparalysis and death. Seven serologically distinct forms of neurotoxin are produced by Clostridium botulinum. The toxin is synthesized as a 150 kDa precursor that is proteolytically nicked into two subunits. The light (L) chain, associated with the toxicity of BoNT, must be internalized in the cell in order to inhibit neurotransmitter release. It is linked by a disulfide bond to the heavy (H) chain, which mediates binding of the toxin to receptors located on the surface of the nerve cell. Although the heavy chain is required for BoNT to productively bind and enter the target cell, it is not toxic by itself.

The current pentavalent toxoid vaccine for botulism is composed of formalin-inactivated holotoxin. Although effective, this vaccine is difficult to manufacture. Furthermore, extensive treatment with formalin is required to inactivate the toxin. Prolonged treatment with formalin can affect the immunogenicity of protein antigens, and this may explain why certain lots of toxoid have been poorly immunogenic in the past.

There are several approaches that can be used to construct a new vaccine. One approach would be to express a non-toxigenic mutant of BoNT/A, as has already been done for other toxins. The advantage of this approach is that the immune response elicited by the modified protein would most closely approximate the response elicited by the native toxin, because almost all of the

TABLE 1. PCR primers

n.t.[a]	Direction[c]	Sequence
367-741	F:	5′-ATATGGAATTCGTTAATAAACAATTTAATTATAAAGATCC-3′ (Seq. ID No. 1)
L_{4-128}[b]	R:	5′-AGTATCGTCGACTTTTAATTCTGTATCTATTGTACTTCCACC-3′ (Seq. ID No. 2)
732-1170	F:	5′-GATACAGAATTCAAAGTTATTGATACTAATAG-3′ (Seq. ID No. 3)
$L_{126-271}$[b]	R:	5′-CTTTGCGTCGACTCCCCCAAATGTTCTAAGTTCC-3 (Seq. ID No. 4)
1126-1750	F:	5′-GGGTTAGAATTCAGCTTTGAGGAACTTAGAACATTTGGG-3′ (Seq. ID No. 5)
$L_{257-465}$[b]	R:	5′-AGGACTGTCGACCAAGTCCCAATTATTAACTTTGATTGATAAATC3′ (Seq. ID No. 6)
1720-2340	F:	5′-TTAAATGAATTCTCAATCAAAGTTAATAATTGGGAC-3′ (Seq. ID No. 7)
$H_{455-661}$[b]	R:	5′-CTCTGGGTCGACTTCTAACAGAATAACAGCTCC-3′ (Seq. ID No. 8)
2150-2780	F:	5′-GAAGTAAGAGCTCTGGATAAAATTGCGGATATAAC-3′ (Seq. ID No. 9)
$H_{630-808}$[b]	R:	5′-TAACCGGTCGACACCATAAGGGATCATAGAG-3′ (Seq. ID No. 11)
2695-3175	F:	5′-GCTATGATTAATATAAATAAATTTTTGAATCAATGC-3′ (Seq. ID No. 10)
$H_{780-939}$[b]	R:	5′-AGTACTAAGCTTTTCATACATACTATTATATACAATAGC-3′ (Seq. ID No. 12)
3100-3530	F:	5′-AAAAATAGAGCTCAATTATTTAATTTAGAAAGTAG-3′ (Seq. ID No. 13)
$H_{915-1059}$[b]	R:	5′-ACCATCGTCGACAAACATTATATTATTACTAGC-3′ (Seq. ID No. 14)
3301-3726	F:	5′-TATGGTGAATTCATCTGGACTTTACAGGATACTCAGG-3′ (Seq. ID No. 15)
$H_{982-1123}$[b]	R:	5′-ATTTACGTCGACATATTTATTTGGATC-3′ (Seq. ID No. 16)
3590-4020	F:	5′-GATAAGGAATTCAATGAAAAAGAAATCAAAG-3′ (Seq. ID No. 17)
$H_{1078-1220}$[b]	R:	5′-CTTCATGTCGACTACTTGACTTAGATTTCC-3′ (Seq. ID No. 18)
3806-4223	F:	5′-AACATTGAATTCAATTCAAGTTTGTATAGGGGG-3′ (Seq. ID No. 19)
$H_{1150-1289}$[b]	R:	5′-TCCATCGTCGACAGGAATAAATTCCCATGAGCTACC-3′ (Seq. ID No. 20)

[a] Nucleotide sequence number designation based on EMBL/Genbank™ accession file X52066.

[b] Amino acid residue number of the light (L) chain and the heavy (H) chain.

[c] F, forward primer; R, reverse primer.

truncated protein.

Protectiv efficacy of BoNT/A proteins. Two weeks after the final immunization, each mouse was challenged i.p. with 2 lethal doses of BoNT/A (2 $MIPLD_{99}$). This dose was chosen for initial screening to observe any potential ability of the proteins to elicit protective immunity. As shown in Table 3, only two proteins protected the majority of animals from death. Both of these fragments were derived from the heavy chain and encoded amino acid residues $H_{455-661}$ and $H_{1150-1289}$.

$H_{455-661}$ of serotype A neurotoxin is the sequence

H_3N-IKVNN WDLFF SPSED NFTND LNKGE EITSD TNIEA AEENI SLDLI QQYYL TFNFD NEPEN ISIEN LSSDI IGQLE LMPNI ERFPN GKKYE LDKYT MFHYL RAQEF EHGKS RIALT NSVNE ALLNP SRVYT FFSSD YVKKV NKATE AAMFL GWVEQ LVYDF TDETS EVSTT DKIAD ITIII PYIGP ALNIG NMLYK DDFVG ALIFS GA-COOH **(Seq. ID No. 21)**

and $H_{1150-1289}$ of serotype A neurotoxin is the sequence

H_3N-LNSSL YRGTK FIIKK YASGN KDNIV RNNDR VYINV VVKNK EYRLA TNASQ AGVEK ILSAL EIPDV GNLSQ VVVMK SKNDQ GITNK CKMNL QDNNG NDIGF IGFHQ FNNIA KLVAS NWYNR QIERS SRTLG CSWEF IPVDD-COOH. **(Seq. ID No. 22)**

Although some of the other truncated proteins appeared to provide partial protection at the challenge dose initially used, none were as definitive as $H_{455-661}$ and $H_{1150-1289}$. Rechallenge of the survivors with 2 $MIPLD_{99}$ of BoNT/A resulted in the death of all mice except those immunized with the two protective fragments. To confirm these results, separate groups of mice were immunized with fragments $H_{455-661}$ and $H_{1150-1289}$ as before and then challenged with 10 $MIPLD_{50}$. The survival rate for mice immunized with $H_{455-661}$ and $H_{1150-1289}$ at this challenge dose was determined to be 87.5% and 60.0%, respectively.